Short report

Population data for DXS6800, DXS101 and DXS8377 loci from Buenos Aires (Argentina)

Pablo A. Noseda BSc, Forensic Expert [a], Jaclyn Kenline MSc, Forensic Expert [b],
Samantha Manning MSc, Forensic Expert [b],
David A. Gangitano PhD, Assistant Professor [b],*

[a] Secretaria Nacional de Ninez, Adolescencia y Familia (SENAF), Laboratorio de Huellas Digitales Geneticas, Paz Soldan 5200,
Buenos Aires C1427DSJ, Argentina
[b] Sam Houston State University, College of Criminal Justice, Department of Forensic Sciences, 1003 Bowers Blvd., Huntsville, TX 77340, USA

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ABSTRACT

The X-chromosomal short tandem repeats (X-STRs) DXS6800, DXS101 and DXS8377 were analysed in a population sample from Buenos Aires (Argentina) using a polymerase chain reaction (PCR) multiplex approach with fluorescent detection. We present allele frequencies for these loci in a population comprising 113 women and 99 men. The Hardy–Weinberg equilibrium (HWE) was tested in the female sample and no significant deviations were observed. The homogeneity of allele frequencies of men and women was compared by the Fisher's exact test and showed similar distributions. Linkage disequilibrium (LD) tests were performed in males for all pairs of loci and no significant associations were detected. Parameters of forensic interest were also estimated.

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1. Introduction

X-chromosomal short tandem repeats (X-STRs) have received great interest from the forensic community due to their usefulness as a complementary tool to autosomal and Y-chromosome markers for human identification. Due to their unique inheritance pattern X-STRs are very helpful in complex kinship cases such as paternity testing involving a female child, maternity testing, cases where only distant relatives are available and incest cases. The main advantage of X-STRs in paternity testing of standard trios involving a female child is the higher mean exclusion chance when compared to autosomal markers with similar polymorphic information content.[1,2] Several population studies on X-chromosome markers have been conducted in Europe,[3] Asia,[4] Africa[5] and America.[6–8] However, further research is necessary to evaluate allele frequencies between different populations across the world and the extent of these polymorphisms to develop a robust reference database for human identification. The aim of this work is to present population

data from Buenos Aires (Argentina) for three X-chromosome markers: DXS6800, DXS101 and DXS8377 and compare their allele-frequency distribution to other populations.

2. Population

A total of 212 unrelated and healthy individuals (113 women and 99 men) residing in the metropolitan area of Buenos Aires city (Argentina) were studied.

3. Methods

3.1. DNA extraction and quantification

Sampling was performed anonymously to prevent linkage to the original donor. Genomic DNA (gDNA) was extracted from blood samples using a modified Chelex® method[9] suspending the resin in TE buffer (Tris–HCl 10 mM, ethylenediamine tetra acetic acid (EDTA) 0.1 mM, pH 8.0). DNA samples were quantified by real-time polymerase chain reaction (PCR) in a StepOne™ Real Time System (Applied Biosystems, Foster City, CA, USA) using 2 μl extracted gDNA, 400 nM D21S11 primers (GenBank Accession number

* Corresponding author. Sam Houston State University, College of Criminal Justice, Department of Forensic Sciences, Office 221C, 1003 Bowers Blvd., Huntsville, TX 77340, USA. Tel.: +1 936 294 4413; fax: +1 936 294 4905.
E-mail address: dag006@shsu.edu (D.A. Gangitano).

AP000433) (Integrated DNA Technologies, Coralville, IA, USA) and $1\times$ SYBR® Green PCR Master Mix (Applied Biosystems). The following real-time PCR cycling parameters were used: 10 min at 95 °C and 40 cycles of 15 s at 95 °C and 1 min at 60 °C.

3.2. Amplification and genotyping

Primers for DXS6800, DXS101 and DXS8377 were designed based on previous studies.[3,4] PCR was performed using 0.5–1 ng gDNA, 0.64 μM FAM-DXS6800, 0.77 μM HEX-DXS8377 and 1.28 μM FAM-DXS101 fluorescent labelled primers (Integrated DNA Technologies), 100 μM deoxyribonucleotidetriphosphates (dNTPs), 0.5× Q solution, 1× PCR buffer and 0.5 units of Hot Start Polymerase (Qiagen, Venlo, The Netherlands). Amplification conditions, using an Eppendorf® Mastercycler (Eppendorf, Hamburg, Germany), consisted of: activation for 15 min at 95 °C, followed by 32 cycles of 45 s at 95 °C, 45 s at 58.8 °C, 45 s at 72 °C and a final extension step of 15 min at 72 °C. Aliquots of 1.5 μl PCR products were mixed with 0.5 μl GeneScan™ LIZ® 500 Size Standard and 24.0 μl Hi-Di™ Formamide (Applied Biosystems) and injected in an ABI PRISM™ 310 Genetic Analyzer (Applied Biosystems). Allele calling was performed using GeneMapper v. 3.7 (Applied Biosystems). A custom-designed bin set was implemented to allow automation of genotyping. A ladder containing all the observed alleles in the population was prepared according to Sajantila et al.[10] The ladder was calibrated with DNA control samples 9947A, 9948 and K562 (Promega, Fitchburg, WI, USA).[11]

3.3. Data analysis

The Hardy–Weinberg equilibrium (HWE) in females and analyses of genetic variation including observed heterozygosity and gene diversity were calculated using the Genetic Data Analysis software.[12] The homogeneity of the allele frequencies of men and women and linkage disequilibrium (LD) in the male sample was assessed using the GENEPOP version 4.1 software package.[13] Allele frequencies and parameters of forensic interest were generated with PowerStats v. 1.2 software.[14] Powers of exclusion calculations were estimated according to Desmarais et al.[15] Population sample comparisons by pair-wise genetic-distance analysis were carried out with Arlequin 3.5 sofware.[16]

3.4. Quality control

The laboratory of the first author successfully participated in proficiency testing of the Latin-American Society of Forensic Genetics 2007 (http://www.slagf.org.ar).

4. Results

Allele frequencies and *p*-values for HWE (females) are shown in Table 1. Fisher's exact test did not reveal allele distribution differences between men and women except for locus DXS6800 ($p = 0.043$). Interestingly, this gender difference in DXS6800 was previously reported in Austrian and German populations.[3]

Table 1
Allele frequencies and Hardy–Weinberg evaluation of three X-chromosome markers in a population sample of Buenos Aires, Argentina (113 women and 99 men, $n = 325$ chromosomes).

Allele	DXS6800			DXS101			DXS8377		
	Females	Males	Combined	Females	Males	Combined	Females	Males	Combined
15				0.0143	0.0208	0.0163			
16	0.5491	0.4848	0.5294	0.0000	0.0104	0.0033			
17	0.0134	0.0505	0.0248	0.0095	0.0104	0.0098			
18	0.1161	0.1313	0.1207	0.0524	0.0417	0.0490			
19	0.2366	0.2222	0.2322	0.0333	0.0313	0.0327			
20	0.0045	0.0303	0.0124	0.0381	0.0313	0.0359			
21	0.0804	0.0707	0.0774	0.0238	0.0104	0.0196			
22	0.0000	0.0101	0.0031	0.0333	0.0208	0.0294			
23				0.0905	0.0417	0.0752			
24				0.1857	0.2083	0.1928			
25				0.2333	0.1771	0.2157			
26				0.1476	0.2188	0.1699			
27				0.0810	0.0938	0.0850			
28				0.0238	0.0521	0.0327			
29				0.0143	0.0208	0.0163			
30				0.0190	0.0104	0.0163			
38							0.0000	0.0103	0.0031
39							0.0044	0.0000	0.0031
40							0.0088	0.0103	0.0093
41							0.0088	0.0103	0.0093
42							0.0752	0.0309	0.0619
43							0.0841	0.0412	0.0712
44							0.0929	0.0825	0.0898
45							0.0752	0.0722	0.0743
46							0.1018	0.1649	0.1207
47							0.1283	0.1340	0.1300
48							0.1283	0.1237	0.1269
49							0.0752	0.0515	0.0681
50							0.0664	0.0825	0.0712
51							0.0442	0.1031	0.0619
52							0.0487	0.0103	0.0372
53							0.0177	0.0309	0.0217
54							0.0177	0.0103	0.0155
55							0.0044	0.0206	0.0093
56							0.0177	0.0103	0.0155
HWE	0.1490			0.5530			0.0770		

HWE: Hardy–Weinberg equilibrium probability values of exact test (3200 shufflings) in females.

Table 2
Parameters of forensic interest of three analyzed X chromosome STR markers.

	DXS6800	DXS101	DXS8377
Ho_f	0.6340	0.8500	0.8100
He_f	0.6240	0.9160	0.8680
PIC_f	0.5740	0.8530	0.9077
PD_f	0.8004	0.9591	0.9808
PD_m	0.6897	0.8587	0.9028
PE trio	0.5998	0.8532	0.9073
PE motherless	0.4521	0.7566	0.8362

Ho_f: observed heterozygosity in females, He_f: expected heterozygosity in females, PIC_f: polymorphic information content in females; PD_f: power of discrimination in females; PD_m: power of discrimination in males; PE: power of exclusion for parentage testing involving a daughter.

Table 3
Population comparison between Buenos Aires (Argentina) and European and Latin-American sample sets by pair-wise genetic-distance analysis, based on F_{st}.

Buenos Aires (Argentina) vs.	DXS6800	DXS101	DXS8377
Tuscany (Italy)[18]	N/C	0.00315 (0.1261)	−0.00083 (0.5225)
Piedmont (Italy)[19]	0.01681 (0.009[a])	0.0006 (0.4054)	0.00097 (0.3333)
Cantabria (Spain)[17]	N/C	0.00181 (0.2883)	0.00253 (0.1802)
Basque Country (Spain)[17]	N/C	−0.00433 (0.8559)	0.0058 (0.099)
Antioquia (Colombia)[21]	−0.00321 (0.5586)	N/C	−0.00286 (0.8198)
Santander (Colombia)[8]	N/C	0.00256 (0.2252)	−0.00109 (0.6306)
Rio Grande do Sul (Brazil)[6]	0.00794 (0.1261)	0.00331 (0.1622)	0.00986 (0.009[a])
Belem (Brazil)[22]	−0.00326 (0.7658)	N/C	N/C

Probability values of F_{st} displayed in parentheses. N/C: not compared.
[a] Statistically significant differences at 0.01 levels.

Nevertheless, after applying Bonferroni correction for multiple tests (significance level, 0.017) no significant differences were found for any loci between male and female samples; therefore their frequencies can be combined. The exact test of pair-wise linkage between markers in males did not detect any evidence of LD ($p > 0.05$). Parameters of forensic interest are displayed in Table 2. Since the Argentinean population is the result of an Amerindian and European (mainly Spaniard and Italian) admixture process, population sample comparisons were carried out with Spanish, Italian, Colombian and Brazilian sample sets[6,8,17–21] by pair-wise genetic-distance analysis based on F_{st}, when the studied markers were available (Table 3). No statistically significant differences were observed except for markers DXS6800, between Argentina and Italy, and DXS8377, when the comparison involved Argentinean and Brazilian population samples. In summary, a population database for markers DXS6800, DXS101 and DXS8377 was developed for forensic and anthropological purposes. The use of these markers along with additional X-STRs should be considered in a forensic scenario, especially in deficiency cases and complex kinship analyses.

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